Remarks

Reconsideration of this Application is respectfully requested.

Claims 2, 3, 48, 49 and 58-62 have been canceled without prejudice to or disclaimer of the subject matter therein. Upon entry of the foregoing amendments, Claims 1, 4-16, 18-47, 50-54 and 57 are pending in the application, with claim 1 being the independent claim. Claims 1, 20-23, 32-37, 39-43, 46, 47, 51, 52, 54 and 57 have been amended. Claims 1, 39-41 and 46 have been amended to further clarify the scope of the claims by reciting that the agent comprises a translocation domain of a clostridial neurotoxin H-chain. Claims 1 and 46 have been further amended to recite that linkage is via a covalent bond. Claim 20 has been made dependent on claim 1 and has been amended to recite that the translocation domain is an H-chain of a clostridial neurotoxin, wherein the H_C domain of the H-chain has been removed or modified. Claim 21 has been amended to recite that the H_C domain has been removed or modified by contacting the H-chain with a derivatising chemical. Corresponding amendments have been made to claims 22 and 23. Claim 32 has been made dependent on claim 1; and claims 32-35 have been amended to recite the translocation domain of a clostridial neurotoxin H-chain thereby ensuring correct antecedent support with claim 1. Claims 36 and 37 have also been made dependent on claim 1, and amended to recite the translocation domain of a clostridial neurotoxin H-chain thereby ensuring correct antecedent support with claim 1. In claims 22, 39, 40, 43 and 57 the term "and/or" has been deleted and replaced by the word "or." The phrase "at least one" has been deleted from claims 22, 37, 40, 42, 43 and 47 and replaced with the word "a" or "an." Claims 51, 52, 54 and 57 have been amended to clarify that administration is to a subject in need thereof, and

by a route selected from the group consisting of intrathecal, subcutaneous and epidural routes. The result of administration (*i.e.*, the outcome of the method) has also been inserted into each of claims 51, 52, 54 and 57. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Rejection under 35 U.S.C. § 112, First Paragraph

The Examiner has rejected claims 1-16, 18-54 and 58-61 under 35 U.S.C. § 112, first paragraph. Office Action, section 4, line 1. Applicants respectfully traverse this rejection.

A. The Examiner's Argument

The Examiner is of the opinion that:

> the specification, while being enabling for an agent (lectin-LH_N) comprising a galactose-binding lectin covalently linked to a fragment of clostridial neurotoxin (LH_N, where L is light chain or its functional fragment, and H_N is a membrane translocation domain), a method for obtaining the agent, or a method of controlling the transmission of sensory information or the sensation of pain by administering the agent, or, for an agent of LH_N linked to lectin, an agent of a modified clostridial neurotoxin having H_C chemically modified to reduce its ability to bind the receptor, linked to lectin, an agent of a hybrid molecule of a modified heavy chain of a clostridial toxin with a light chain of a different clostridial toxin, linked to lectin, or a fusion protein of the agent expressed recombinantly as indicated in the prior art, does not reasonably provide enablement for an agent comprising a galactose-binding lectin or a modified galactose-binding lectin, the L chain of a clostridial toxin

> or its functional fragment, and a membrane translocation domain, wherein the three components are linked together, a method for obtaining the agent and a method of controlling the transmission of sensory information or the sensation of pain by administering the agent. The specification does not enable a person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Office Action, section 4, lines 2-17. Applicants respectfully disagree.

The Examiner phrases this rejection in more specific terms when he states that:

> the specification needs to provide specific guidance on
> [1] the modified galactose-binding lectin,
> [2] the membrane translocation domain, and
> [3] the linkage of three components
> to be considered to be enabling for variants.

Office Action, section 4, lines 51-54 (numbering and indentations added). Applicants respectfully disagree.

1. "[T]he modified galactose-binding lectin"

Claim 1 is directed to "[a]n agent, for the treatment of pain, that comprises:- a galactose-binding lectin; . . . " Claims 4-16, 18-47 and 50-54 are directly or indirectly dependent upon claim 1. Claims 2, 3, 48, 49 and 58-62 have been canceled. None of the claims that the Examiner has rejected under 35 U.S.C. § 112, first paragraph, recites a "modified galactose-binding lectin" *verbatum* or otherwise. Adding an extraneous limitation into a claim is improper. *Datascope Corp. v. SMEC, Inc.*, 879 F.2d 820, 824, 11 USPQ2d 1321, 1323 (Fed. Cir. 1989), *cert. denied*, 110 S. Ct. 729 (1990); *Corning Glass Works v. Sumitomo Electric U.S.A.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1967 (Fed. Cir. 1989); *E.I. du Pont de Nemours & Co. v. Phillips Petroleum Co.*, 849 F.2d 1430, 1433, 7 USPQ2d 1129,

1131 (Fed. Cir. 1988), *cert. denied*, 488 U.S. 986. Therefore, none of claims 1-16, 18-54 and 58-61 lack enablement under 35 U.S.C. § 112, first paragraph, in view of the phrase "the modified galactose-binding lectin."

2. "[T]he membrane translocation domain"

Claims 2, 3, 48, 49 and 58-62 have been canceled. None of claims 1, 4-16, 18-47 and 50-54 recite the phrase "membrane translocation domain." Claim 1 does recite the phrase "a translocation domain of a clostridial neurotoxin H-chain." Claims 4-16, 18-47 and 50-54 are directly or indirectly dependent upon claim 1. The working examples provided in the specification, which demonstrate the preparation of ExL-LH_N/A, EcL-LH_N/A and SBA-LH_N/A, provide all the necessary guidance for one of ordinary skill in the art to carry out the present invention without undue experimentation.

Furthermore, the term "translocation domain of a clostridial neurotoxin H-chain"is a term of art, and is recognized as such by those of ordinary skill in the study of clostridial neurotoxins. The "translocation domain of a clostridial neurotoxin H-chain" is simply that portion of the H-chain that allows translocation of an L-chain or a functional fragment thereof into the target cell. Prior to the present invention, a number of simple tests were readily available to confirm whether a particular portion of a clostridial toxin H-chain possess the necessary "translocation" function. *See, e.g.,* Shone, C.C., *et al.*, "A 50-kDa fragment from the NH_2-terminus of the heavy subunit of Clostridium botulinum type A neurotoxin forms channels in lipid vesicles," *Eur J. Biochem. 167*:175-180 (1987) (Shone *et al.* II) and Blaustein, R.O., *et al.*, "The N-terminal half of the heavy chain of botulinum type A

neurotoxin forms channels in planar phospholipid bilayers," *FEBS Letters 226*:115-120 (1987) (Blaustein *et al.*). Copies of Shone *et al.* II and Blaustein *et al.* are submitted herewith as Documents AT8 and AR8, respectively, in a Supplemental Information Disclosure Statement. A summary of these published methods is attached hereto as Appendix A. By following these simple tests, it would be routine for one of ordinary skill in the art to determine whether a given H-chain portion possesses the requisite "translocation" function. Therefore, one of ordinary skill in the art can practice the present invention without undue experimentation. Thus, claim 1 and each of its dependent claims is fully enabled.

3. "[T]he linkage of three components"

Claims 2, 3, 48, 49 and 58 have been canceled. In order to more clearly describe Applicants' claimed invention, claims 1 and 46 have been amended to recite that linkage is *via* a covalent bond.

The agent of claim 1 comprises three components, which for the purposes of this discussion are referred to a Domains "B," "T" and "E." Domain B (binding) is the galactose-binding lectin, Domain T (translocation) is the translocation domain of a clostridial neurotoxin H-chain and Domain E (effector) is the L-chain or L-change fragment of a clostridial neurotoxin. Claim 1 is directed to an agent *comprising* Domain B, Domain T and Domain E. The functionality of the claimed agent is unaffected by the order in which these three domains are linked. Furthermore, the illustrated conjugation method results in the production of mixed conjugates. Thus, the invention is not restricted to agents having a

specific linkage order. For the convenience of the Examiner an exemplary list of combinations falling within the scope of claim 1 is appended hereto as Appendix B.

There is no statutory requirement that a claim contain unnecessary limitations. The individual components of the agent of claim 1 are clearly defined. The means by which the individual components are attached (a covalent bond) is clearly defined. The order of attachment is immaterial. Therefore, the order of attachment need not be recited in claim 1. Consequently, claim 1 and its dependent claims are enabled.

B. The Examiner's Rebuttal

In the previous Office Action (Paper No. 9, mailed on August 14, 2001), the Examiner rejected claims 1-27 and 32-54 under 35 U.S.C. § 112, first paragraph. *See* Paper No. 9, section 2. Applicants responded in an Amendment and Reply that was filed on December 14, 2001 (*see* page 13, line 1, through page 32, line 5).

The present Office Action contains the Examiner's rebuttal (*see* section 4, lines 94-109). Applicants respectfully disagree with the Examiner's rebuttal. Each of the points raised in this rebuttal has been addressed in sections I.A.1-3, above. Applicants respectfully submit that the Examiner's rebuttal is insufficient to sustain the present rejection of claims 1-16, 18-54 and 58-61 under 35 U.S.C. § 112, first paragraph.

C. Summary

Applicants respectfully submit that the rejection of claims 1-16, 18-54 and 58-61 under 35 U.S.C. § 112, first paragraph, has been overcome and should be withdrawn.

II. Rejections under 35 U.S.C. § 112, Second Paragraph

A. The First Rejection

The examiner has rejected claims 1-16, 18-54 and 58-61 under 35 U.S.C. § 112, second paragraph, as being indefinite. Office Action, section 2, lines 1-3. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that:

> Claims 1-16, 18-54 and 58-61 are indefinite [] because of the use of the term "wherein the galactose-binding lectin, L-chain or fragment, and molecule or domain with membrane translocating activity are linked together". The term "wherein the galactose-binding lectin, L-chain or fragment, and molecule or domain with membrane translocating activity are linked together" renders the claim indefinite, it is unclear how the three components are linked, e.g., is the N-terminus of lectin linked to the N-terminus of the peptide including L-chain and H_N chain (lectin-LH_N), or the N-terminus of lectin linked to the N-terminus of H_N chain and then to the L-chain (lectin-H_N-L), or the L-chain linked to lectin and then to H_N chain (L-lectin-H_N)? The specification indicates SPDP (a linking agent for amino group) is used for linking ExL and [LH_N/A] (Example 1, page 13), however, it does not specify which amino group (at N-terminus of lysine side chain) in the peptide is modified by SPDP, thus it is not apparent how the two peptides are linked. Claims 2-16, 18-54 and 58-61 are included in the rejection for being dependent of a rejected claim and not correcting the deficiency of the claim from which they depend.

Office Action, section 5, lines 4-17. Applicants respectfully disagree.

Claims 2, 3, 48, 49 and 58-61 have been canceled. In order to more clearly describe Applicants' claimed invention, claims 1 and 46 have been amended to recite that linkage is *via* a covalent bond.

The agent of claim 1 comprises three components, which for the purposes of this discussion are referred to a Domains "B," "T" and "E." Domain B (binding) is the galactose-binding lectin, Domain T (translocation) is the translocation domain of a clostridial neurotoxin H-chain and Domain E (effector) is the L-chain or L-change fragment of a clostridial neurotoxin. Claim 1 is directed to an agent *comprising* Domain B, Domain T and Domain E. The functionality of the claimed agent is unaffected by the order in which these three domains are linked.

There is no statutory requirement that a claim contain unnecessary limitations. The individual components of the agent of claim 1 are clearly defined. The means by which the individual components are attached (a covalent bond) is clearly defined. The order of attachment is immaterial. Therefore, the order of attachment need not be recited in claim 1. Consequently, claim 1 and its dependent claims are not indefinite. Applicants respectfully submit that the rejection of claims 1-16, 18-54 and 58-61 under 35 U.S.C. § 112, second paragraph, has been overcome and should be withdrawn.

B. Second Rejection

The Examiner has rejected claims 2, 20-24, 32-34 and 48 under 35 U.S.C. § 112, second paragraph, as being indefinite. *See* Office Action, section 6, line 1. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that

> Claims 2, 20-24, 32-34 and 48 are indefinite because the claim[s] do[] not further limit the claim (claim 1) which they depend from, the membrane translocation domain is part of the heavy chain of a clostridial toxin, thus it is narrower than the parent peptide. Claims 20-24, 32-34 and 48 are included in the rejection for being dependent of a rejected claim and not correcting the deficiency of the claim from which they depend.

Office Action, section 6, lines 1-5. Applicants respectfully disagree.

Claims 2 and 48 have been canceled. Claims 20-24 and 32-34, as amended, depend directly or indirectly on claim 1. As is demonstrated herein (*see, e.g.,* sections I.A, II.A and III), claim 1 is allowable. Therefore, claims 20-24 and 32-34 are each based upon an allowable base claim. Applicants respectfully submit that the rejection of claims 2, 20-24, 32-34 and 48 under 35 U.S.C. § 112, second paragraph, has been overcome and should be withdrawn.

C. The Third Rejection

The Examiner has rejected claims 2, 32-34 and 48 under 35 U.S.C. § 112, second paragraph, as being indefinite. *See* Office Action, section 6, line 6. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that:

> Claims 2, 32-34 and 48 are also indefinite because of the use of the term "the membrane translocation domain is a heavy chain of a clostridial toxin. The term "the membrane translocation domain is a heavy chain of a clostridial toxin" renders the claim indefinite, it is unclear how the agent having both galactose-binding lectin as a targeting moiety and H_C component, a native targeting moiety, binds to the motor neurons?

Office Action, section 6, lines 6-10. Applicants respectfully disagree.

Claims 2 and 48 has been canceled. Claims 32-34, as amended, depend directly or indirectly on claim 1. Claim 1 does not recite the phrase "the membrane translocation domain is a heavy chain of a clostridial toxin." Claim 1 does recite the phrase "a translocation domain of a clostridial neurotoxin H-chain," which is not indefinite. Therefore none of claims 32-34 is indefinite. Applicants respectfully submit that the rejection of claims 2, 32-34 and 48 under 35 U.S.C. § 112, second paragraph, has been overcome and should be withdrawn.

D. The Fourth Rejection

The Examiner has rejected claims 20-23 under 35 U.S.C. § 112, second paragraph, as indefinite. *See* Office Action, section 7, line 1. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that:

> Claims 20-23 are indefinite because of the use of the term "modified to remove or reduce the native binding affinity of the H-chain for motor neurons", "H-chain has been contacted with a derivatising chemical to.....neurons", "H-chain has been mutated by the inclusion of at least one amino acid deletion, insertion, and/or substitution...neurons", or "H-chain has been contacted with a proteolytic agent....neurons". The terms cited above render the claim indefinite, it is unclear how H-chain is modified to reduce the native binding affinity of the H-chain for motor neurons, what compound is used for the modification as to "a derivatising chemical" or "proteolytic agent", and how many amino acids and which residues are deleted, inserted or substituted as to "mutated by the inclusion of at least one amino acid deletion, insertion, and/or substitution". See also claims 42 and 43 as to "at least one amino acid insertion, deletion, or substitution" or "at least one nucleotide deletion, insertion, and/or substitution".

Office Action, section 7, lines 1-12. Applicants respectfully disagree.

1. "[M]odified to remove or reduce the native binding affinity of the H-chain for motor neurons"

As clearly indicated in claims 20-23, a modified H-chain will fall within the scope of the present invention if the modification results in a loss of or reduction in the native binding affinity of the H-chain for its natural target cells, *i.e.*, motor neurons. Binding of the H-chain to motor neurons may be assessed by assays well-known to those of ordinary skill in the art. For examples see, Shone, C.C., *et al.*, "Inactivation of Clostridium botulinum type A neurotoxin by trypsin and purification of two tryptic fragments," *Eur. J. Biochem. 151*:75-82 (1985) (Shone *et al.* I); Sutton, J.M., *et al.*, "Tyrosine-1290 of tetanus neurotoxin plays a key role in its binding to gangliosides and functional binding to neurones," *FEBS Letters 493*:45-49 (2001) (*see* page 46, col. 2, lines 6-21, wherein the authors attributes the method of the binding assay to Shone *et al.* I) (Sutton *et al.*); Black, J.D., and Dolly, J.O., "Interaction of 125I-labeled botulinum neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves," *J. Cell Biol 103*:521-534 (1986) (Black and Dolly). Copies of Shone *et al.* I, Sutton *et al.*, and Black and Dolly are submitted herewith as Documents AS8, AR9, and AT7, respectively, in a Supplemental Information Disclosure Statement. A summary of these published methods is attached hereto as Appendix C.

Methods for removing or modifying the H_C domain of a clostridial neurotoxin H-chain by contacting with a derivatising chemical are well known to those of ordinary skill in the art, and the appropriate chemicals are readily from standard laboratory chemical catalogs. These chemicals include, but are not limited to:

A. tyrosine derivatising chemicals such as anhydrides, more specifically maleic anhydride;

B. diazonium based derivatising chemicals such as *bis*-diazotized *o*-tolidine and diazotized *p*-aminobenzoyl biocytin;

C. EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride);

D. isocyanate based derivatising chemicals such as dual treatment with tetranitromethane followed by sodium dithionite; and

E. iodinating derivatising chemicals such as chloramine-T (N-chlorotoluene sulfonamide) or IODO-GEN (1,3,4,6-tetrachloro-3α,6α-diphenylglycouril).

Methods for removing or modifying the H_C domain of a clostridial neurotoxin H-chain by contacting with a proteolytic agent are also well known to those of ordinary skill in the art. Suitable proteolytic agents include, but are not limited to:

A. trypsin (*see, e.g.,* Shone *et al.* I);

B. proline endopeptidase;

C. lys C proteinase;

D. Chymotrypsin;

E. thermolysin; and

F. arg C proteinase.

Thus, claims 20-23 are not indefinite.

2. "[A]t least one"

Claims 22 and 42 have been amended to delete the phrase " at least one amino acid" and to insert the phrase "an amino acid" therefor. Claim 43 has been amended to delete the phrase "at least one nucleotide" and to insert the phrase "a nucleotide" therefor. Applicants respectfully submit that this stated grounds for rejection has been accommodated and that amended claims 22, 42 and 43 are not indefinite.

3. "[A]nd/or"

Claims 22 and 43 have been amended to delete the term "and/or" and to insert the word "or" therefor. Applicants respectfully submit that this stated grounds for rejection has been accommodated and that amended claims 22 and 42 are not indefinite.

4. Summary

Applicants respectfully submit that all of the stated grounds for the rejection of claims 20-23 under 35 U.S.C. § 112, second paragraph, have been overcome and the rejection should be withdrawn.

E. The Fifth Rejection

The Examiner has rejected claims 22, 39, 40 and 43 under 35 U.S.C. § 112, second paragraph, as being indefinite. *See* Office Action, section 8, line 1. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that "[claims] 22, 39, 40 and 43 are also indefinite because of the use of the term 'and/or'. The term 'and/or' renders the claim indefinite, it is unclear whether the limitation after 'and/or' is included or not, and if included is to be read as an alternative 'or' or the conjunctive 'and'." Office Action, section 8, lines 1-3.

Claims 22, 39, 40 and 43 have been amended such that the term "and/or" does not appear. Applicants respectfully submit that the rejection of claims 22, 39, 40 and 43 under 35 U.S.C. § 112, second paragraph, has been rendered moot and should be withdrawn.

F. The Sixth Rejection

The Examiner has rejected claims 37, 40 and 47, under 35 U.S.C. § 112, second paragraph, as being indefinite. *See* Office Action, section 9, line 1. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that "[c]laims 37, 40 and 47 are indefinite because of the use of the term 'at least one spacer region'. The term 'at least one spacer region' renders the claim[s] indefinite, it is unclear how many spacer regions are between two components." Office Action, section 9, lines 1-3. Applicants respectfully disagree.

Claims 37, 40 and 47 have each been amended to delete the phrase "at least one spacer region" and to insert the phrase "a spacer region" therefor. Applicants respectfully submit that the rejection of claims 37, 40 and 47 under 35 U.S.C. § 112, second paragraph, has been accommodated and should be withdrawn.

G. The Seventh Rejection

The Examiner has rejected claims 51-54 and 58-61 under 35 U.S. C. § 112, second paragraph, as being indefinite. *See* Office Action, section 10, line 1. Applicants respectfully traverse this rejection.

Specifically, the Examiner is of the opinion that

> Claims 51-54 and 58-61 are indefinite because they lack essential steps as claimed in the process of controlling the transmission of sensory information. The omitted steps are: the site and method for administration, the subject receiving the administration of the agent and a step whereby the desired outcome can be determined.
>
> In response, applicants indicate the effective amount has been added in the claim, and the method of administration has been added in claims 58-61. However, claims 51-54 do not cite the method of administration and the outcome of method.

Office Action, section 10, lines 1-7. Applicants respectfully disagree.

Claims 58-61 have been canceled. Without acquiescing to the propriety of the Examiner's rejection, claims 51, 52 and 54 have been amended to clarify that administration is to a subject in need thereof and by a route selected from the group consisting of intrathecal, subcutaneous and epidural routes. The result of administration (*i.e.*, the "outcome" of the method) has also been inserted into each of claims 51, 52 and 54. Claim 53 is dependent

upon claim 51. Applicants respectfully submit that the rejection of claims 51-54 and 58-61 under 35 U.S. C. § 112, second paragraph, has been accommodated and should be withdrawn.

III. Rejection under 35 U.S.C. § 102

The Examiner has rejected claims 1, 2, 4-16, 20-41, 44-48 and 50-54 under 35 U.S.C. § 102(b) as being anticipated by Foster *et al.* (WO 96/33273). Office Action, section 11, lines 1-2. Applicants respectfully traverse this rejection.

A. The Examiner's Argument

Specifically, the Examiner is of the opinion that

> Foster *et al.* teach an agent containing lectin (page 13, lines 9-13) as the TM component and a modified clostridial neurotoxin such as LH_N (including L-chain and its functional fragment, claims 1, 4-16, 24-31, 35, 39, 46, 50), the clostridial neurotoxin having H_C chemically modified to reduce its ability to bind the receptor (claims 2, 20-23, 36, 48), a hybrid molecule of a modified heavy chain (H_C being modified) of a clostridial toxin with a light chain of a different clostridial toxin (page 13, line 18-page 14, line 19; claims 32-34) can be obtained by covalently attachment of a TM to a modified clostridial neurotoxin using linkage including one or more spacer regions (page 14, lines 1-9; claim 37, 40, 47) or can be expressed recombinantly as a fusion protein (page14, line 29-page 15, line 4; claim[s] 38, 41, 50). This agent can bind to a binding site on the surface of sensory neurons (page 12, lines 25-28) and reduce and preferably prevent the transmission of pain signals from nociceptive afferents to projection neurons (page 7, lines 15-17; claims 44-45), therefore it can be used for controlling the transmission of sensory information or pain signals from a nociceptive afferent to a projection neuron (claims 51-54). The agent can be administered by epidural or intrathecal injection (page 16, lines 12-15; claims 58-61).

> Note that the lectin indicated in the reference of Foster *et al.* is generic to a plurality of different species, thus it would include the lectin that binds galactose.

Office Action, section 11, lines 3-19. Applicants respectfully disagree. Claims 2 and 48 have been canceled.

1. The Examiner has not established a prima facie case of anticipation

"Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic & Research Foundation v. Genentech Inc.*, 927 F.2d 1565, 1576, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991) (citations omitted). *See also Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1015, 1053 (Fed. Cir. 1987) ("A claim is anticipated only if each and every element as set fourth in the claim is found, either expressly or inherently described, in a single prior art reference."); *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989) ("The identical invention must be shown in as complete detail as is claimed in the . . . claim."). The Examiner does not dispute the fact that Foster *et al.* do not specifically disclose a galactose-binding lectin. Claim 1 of the present invention is directed to "[a]n agent, for the treatment of pain, that comprises:- a galactose-binding lectin . . . ." All of the other pending claims are dependent, directly or indirectly, on claim 1. Thus, Foster *et al.* do not disclose an element as set forth in the pending claims: a galactose-binding lectin. Therefore, Foster *et al.* do not anticipate any of claims 1, 2, 4-16, 20-41, 44-48 and 50-54.

The Examiner attempts to support an anticipation rejection by noting that "the lectin indicated in the reference of Foster *et al.* is generic to a plurality of different species, thus it would include the lectin that binds galactose." Office Action, section 11, lines 18-19. Applicants respectfully disagree.

A claim to a species is not anticipated by prior disclosure of a genus or class including that species unless disclosure actually names or clearly points to the claimed species. *In re Schaumann*, 572 F.2d 312, 197 USPQ 5 (CCPA 1978)). Anticipation in genus-species situations is discussed in the M.P.E.P., eighth edition, August 2001, at § 2131.02. This section is directed primarily to chemical genera described by structural formulae in Markush-style claims. However, the principles of the cases cited therein are relevant to this rejection. In a genus-species situation, anticipation can only be found "[i]f one of ordinary skill in the art is able to 'at once envisage' the specific [claimed] compound within the [disclosed] generic chemical formula." *Id.* at page 2100-71, col. 1, lines 40-43 (quoting *In re Petering*, 301 F.2d 676, 681, 133 USPQ 275, 280 (CCPA 1962)). It follows that an anticipatory genus must be both well delineated and limited in size. *See In re Petering.*

The Examiner does not contend that Foster *et al.* discloses galactose-binding lectin. Furthermore the Examiner has not demonstrated that Foster *et al.* clearly points to galactose-binding lectin. Therefore, the Examiner has not established a *prima facie* case that Foster *et al.* anticipates any of claims 1, 2, 4-16, 20-41, 44-48 and 50-54.

The Examiner has not asserted that one of ordinary skill in the art at the time the invention was made would read Foster *et al.* and "at once envisage" galactose-binding lectin. The Examiner has not provided any reason why on of ordinary skill in the art at the time the invention was made would read Foster *et al.* and, from the broad genus of Targeting Moieties

disclosed, would have preferentially selected lectins as a sub-genus for further consideration. Furthermore, the Examiner has presented no information showing that the "lectins" disclosed in Foster *et al.* represents a sub-genus that (1) is well delineated, (2) is limited in size and (3) comprises galactose-binding lectins. Therefore, the Examiner has not established a *prima facie* case that Foster *et al.* anticipates any of claims 1, 2, 4-16, 20-41, 44-48 and 50-54.

2. The Examiner's anticipation rejection is contrary to the facts and precluded by the applicable case law

Foster *et al.* teaches an agent for the modification of peripheral sensory afferent functions comprising (a) a clostridial neurotoxin, or a hybrid of two clostridial neurotoxins, in which the H_C region of the H-chain has been removed or modified, which is covalently linked to a "Targeting Moiety (TM)", that binds to a binding site (BS) on the surface of sensory neurons, wherein the agent is "capable of inhibiting the release of at least one neurotransmitter or neuromodulator from nociceptive afferents." Foster *et al.*, page 12, line 25, through page 13, line 1.

A Targeting Moiety (TM) is defined as "any chemical structure of an agent which functionally interacts with a binding site causing a physical association between the agent and the surface of a primary sensory afferent." Foster *et al.*, page 9, lines 16-18. Foster *et al.* further specify that "[t]he TM component of the agent can comprise one of many cell binding molecules, including, but not limited to, antibodies, monoclonal antibodies, antibody fragments (Fab, $F(ab)'_2$, Fv, ScFv, etc.), lectins and ligands to the receptors for hormones, cytokines, growth factors or neuropeptides" (*see* page 13, lines 9-13). Foster *et al.* do not disclose any specific suitable lectins. However, in Table 1 at pages 24 and 25, Foster *et al.*

do disclose several possibly suitable antibodies, monoclonal antibodies, ligands to the receptors for cytokines, ligands to the receptors for growth factors, and ligands to the receptors for neuropeptides, as follows:

antibodies: (1) antibodies against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons, (2) antibodies against any of the receptors listed in Table 1 of Foster *et al.* and (3) antibodies against the surface expressed antigen Thy1;

monoclonal antibodies: (1) monoclonal antibodies against the lactoseries carbohydrate epitopes found on the surface of dorsal root ganglion neurons (e.g., monoclonal antibodies 1B2 and LA4) and (2) monoclonal antibodies against the surface expressed antigen Thy1 (e.g., monoclonal antibody MRC OX7);

ligands to the receptors for cytokines: (1) tumor necrosis factor (TNF-), (2) Interleukin-1 (IL-1) and (3) Interleukin-8 (IL-8);

ligands to the receptors for growth factors: (1) nerve growth factor (NGF), (2) leukaemia inhibitory factor (LIF), (3) basic fibroblast growth factor (bFGF), (4) brain-derived neurotrophic factor (BDNF), (5) neurotropin-3 (NT-3), (6) hydra head activator peptid (HHAP), (7) transforming growth factor 1 (TGF-1), (8) transforming growth factor 2 (TGF-2), (9) transforming growth factor (TGF-), (10) epidermal growth factor (EGF) and (11) ciliary neuro-trophic factor (CNTF); and

ligands to the receptors for neuropeptides: (1) endorphin, (2) methionine-enkephalin, (3) D-Ala^2-D-Leu^5-enkephalin and (4) bradykinin.

Thus, the agents disclosed in Foster *et al.* each bind to any one of hundreds or possibly thousands of binding sites. Since the number of ligands that are capable of binding to each individual binding site is potentially vast, it follows that the Targeting Moiety disclosed in Foster *et al.* represent a genus consisting of a nearly infinite number of individual chemical compounds. Furthermore, since the potential binding sites disclosed in Foster *et al.* are extremely diverse, it follows that the Targeting Moiety disclosed in Foster *et al.* represents a genus of highly diverse individual chemical compounds.

Applicants respectfully submit that the Examiner's finding of anticipation for an unnamed species in the midst of such a vast genus is contrary to applicable case law. *See, e.g., In re Meyer*, 599 F.2d 1026, 202 USPQ 175 (CCPA 1979) (A reference disclosing "alkaline chlorine or bromine solution" embraces a large number of species and cannot be said to anticipate claims to "alkali metal hypochlorite.").

Assuming, *arguendo*, (1) that Foster *et al.* provides some motivation for one of ordinary skill in the art at the time the invention was made to consider the broad Targeting Moiety genus disclosed in Foster *et al.* and to preferentially select lectins for further consideration and (2) that the sub-genus of lectins disclosed in Foster *et al.* does comprise a plurality of different species including galactose-binding lectins, the Examiner's finding of anticipation is still incorrect. It is well known to those of ordinary skill in the art that lectins represent a group of compounds that is very large in number. At least 70 lectins have been isolated from leguminous plants alone. Sharon, N., and Lis, H., "Legume lectins – a large family of homologous proteins," *FABS Journal 4*:3198-3208 (1990) (Sharon and Lis). A copy of Sharon and Lis was submitted previously as Document AT4 in a Supplemental Information Disclosure Statement. In addition to this plant family, lectins are also widely

distributed in animals, insects, non-legume plants and microorganisms. *Id.* at page 3198, col. 1, text lines 1-4. The legume lectins "very markedly in their specificity." *Id.* at page 3199, col 1, lines 8-9; and Table 1.

Additionally, the number of potential lectin targets is exceedingly large. The magnitude of these targets can be appreciated by reference to Yamazaki, N., *et al.*, "Endogenous lectins as targets for drug delivery," *Advances in Drug Discovery Reviews 43*:225-244 (2000) (Yamazaki *et al.*). A copy of Yamazaki *et al.* is submitted herewith as Document AS9 in a Supplementary Information Disclosure Statement. Yamazaki *et al.* discuss the concept of the "sugar code" and disclose that due to the diverse range of sugar monomers, modification and multimetric organization, a vast number of glyco-structures may exist in nature. Therefore, the range of specificity for glyco-binding lectins may be equally vast.

It follows that galactose-binding lectins represent a very small sub-set of a large and extremely diverse lectin sub-genus within the Targeting Moiety genus. Thus, one of ordinary skill in the art at the time the invention was made *could not* read Foster *et al.* and "at once envisage" galactose-binding lectin. Applicants respectfully submit that the Examiner's finding of anticipation is precluded by applicable case law. *See, e.g., In re Meyer.*

Furthermore, "[o]ne may look to the preferred embodiments to determine which compounds can be anticipated." M.P.E.P., eighth edition, August 2001, page 2100-71, col. 2, lines 1-3 (*citing In re Petering*). Foster *et al.* disclose a large number of potential targeting moieties (*see* page 13, lines 9-17) and present an illustrative list of possible targeting moieties in Table 1 (pages 24-25). Lectins do not appear in this illustrative list. Furthermore, the examples are directed exclusively to agents wherein the Targeting Moiety is "derivatized"

neural growth factor. *See* Foster *et al.*, Examples 1-3, at page 16, line 18, through page 21, line 22. Thus, Foster *et al.* should be regarded as disclosing the genus of "targeting moieties that bind to peripheral sensory afferents" and lectins should be further regarded as a diverse and non-preferred sub-genus within this genus. Assuming, *arguendo*, that the Examiner does meet the Examiner's evidentiary burden of documenting that galactose-binding lectins are within the scope of Foster *et al.*, then galactose-binding lectins would be yet a *further* subset within the "lectins" sub-genus. One of ordinary skill in the art at the time the invention was made *could not* read Foster *et al.* and "at once envisage" galactose-binding lectin. From looking at the preferred embodiments of Foster *et al.*, the use of a galactose-binding lectin could not be anticipated. Therefore, Foster *et al.* does not anticipate any of claims 1, 2, 4-16, 20-41, 44-48 and 50-54 of the present invention.

B. The Examiner's Rebuttal

In the previous Office Action (Paper No. 9, mailed on August 14, 2001), the Examiner rejected claims 1, 2, 4-48 and 50-54 under 35 U.S.C. § 102(a) as anticipated by Foster *et al. See* Paper No. 9, section 13. Applicants responded in an Amendment and Reply that was filed on December 14, 2001 (*see* page 39, line 3, through page 40, line 4).

In the present Office Action, the Examiner's rebuttal is as follows:

> In response, applicants indicate Foster *et al.* do not teach a specific targeting moiety such as a galactose-binding lectin. The argument is not found persuasive because Foster *et al.* disclose lectin as a generic term which would include different species of lectin, and it appears that the conjugate of lectin and modified clostridial toxin has the same function as the agent of the present invention.

Office Action, section 11, lines 20-24. Applicants respectfully disagree.

The Examiner's argument is legally insufficient to support a *prima facie* case of anticipation for the reasons given in section III.A, above. Furthermore, the Examiner's assertion that the conjugate of lectin and modified clostridial toxin has the same *function* as the agent of the present invention, whether factually true or not, is legally irrelevant with regard to at least claims 1, 2, 4-16 20-41 and 40-48. The unclaimed properties of a composition of matter are irrelevant to anticipation. *See In re Petering* at 682, 133 USPQ at 280. Applicants respectfully submit that the Examiner's rebuttal is insufficient to sustain the present rejection of claims 1, 2, 4-16, 20-41, 44-48 and 50-54 under 35 U.S.C. § 102(a).

C. Summary

For the reasons given above, Applicants respectfully submit that the rejection of claims 1, 2, 4-16, 20-41, 44-48 and 50-54 under 35 U.S.C. § 102(a) has been overcome and should be withdrawn.

IV. Election/Restriction

In Paper No. 6, mailed March 1, 2001, the Examiner required that the claims be restricted to one of four groups. It appears that this restriction was erroneously based upon the claims as printed in the corresponding international publication (WO 99/17806) because the restriction requirement encompasses a total of 61 claims. *See* Paper No. 6, Office Action Summary, sections 4 and 8.

In the Reply to Restriction Requirement, filed on April 3, 2001, Applicants provisionally elected the subject matter of claims 1-45. Applicants also requested that the Examiner base his examination upon the pending claims which were the claims present in the Annexes to the International Preliminary Examination Report as amended by a Preliminary Amendment that was filed on April 7, 2000. In the first Office Action on the merits of the application (Paper No. 9, mailed August 14, 2001), the Examiner rejoined claims 46-54 to claims 1-45. As a consequence, claim 57 is presently withdrawn from prosecution. *See* Paper No. 14, Office Action Summary, section 4(a).

Claim 57, as amended herein, reads as follows:

> 57. (Twice amended) A method of alleviating or preventing pain which comprises administering to a subject in need thereof an effective dose of the agent according to Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus alleviating or preventing pain.

Applicants respectfully submit that claim 57 is directed to the elected subject matter of the present application. As an example of the subject matter of the present application, pending claim 54, as amended herein, reads as follows:

> 54. (Thrice amended) A method of controlling the sensation of pain by administering to a subject in need thereof an effective amount of the agent of Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus controlling the sensation of pain.

Applicants respectfully request that claim 57 be rejoined for prosecution in the present application.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

In the Claims:

Claims 2, 3, 48, 49 and 58-62 have been canceled.

Claims 1, 20-23, 32-37, 39-43, 46, 47, 51, 52, 54 and 57 have been amended as follows:

1. (Twice amended) An agent, for the treatment of pain, that comprises:- a galactose-binding lectin; a light (L) chain or an L-chain fragment of a clostridial neurotoxin, which L-chain or L-chain fragment includes the active proteolytic enzyme domain of the L-chain; and a [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain; wherein the galactose-binding lectin, L-chain or L-chain fragment, and [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain are linked together by a covalent bond.

20. (Thrice amended) An agent according to Claim [2] 1, wherein the translocation domain is an H-chain of a clostridial neurotoxin, wherein the H_C domain of the H-chain has been removed or modified to remove or reduce the native binding affinity of the H-chain for motor neurons.

21. (Thrice amended) An agent according to Claim 20, wherein the H_C domain has been removed or modified by contacting the H-chain [has been contacted] with a derivatising chemical to [reduce or remove] remove or reduce the native binding affinity of the H-chain for motor neurons.

22. (Thrice amended) An agent according to Claim 20, wherein the H_C domain has been removed or modified by mutating the H-chain [has been mutated] by the inclusion of [at least one] an amino acid deletion, insertion, [and/or] or substitution to [reduce or remove] remove or reduce the native binding affinity of the H-chain for motor neurons.

23. (Thrice amended) An agent according to Claim 20, wherein the H_C domain has been removed or modified by contacting the H-chain [has been contacted] with a proteolytic agent to [reduce or remove] remove or reduce the native binding affinity of the H-chain for motor neurons.

32. (Thrice amended) An agent according to Claim [2] 1 in which the translocation domain of a clostridial neurotoxin H-chain has been obtained from a different clostridial neurotoxin than that from which the L-chain or a fragment thereof has been obtained.

33. (Twice amended) An agent according to Claim 32 in which the translocation domain of a clostridial neurotoxin H-chain has been obtained from botulinum neurotoxin type A and the L-chain or a fragment thereof from botulinum neurotoxin type B.

34. (Twice amended) An agent according to Claim 32 in which the translocation domain of a clostridial neurotoxin H-chain has been obtained from botulinum neurotoxin type A and the L-chain or a fragment thereof from tetanus neurotoxin.

35. (Twice amended) An agent according to Claim 33 in which the translocation domain of a clostridial neurotoxin H-chain component is the H_N fragment of botulinum neurotoxin type A.

36. (Thrice amended) An agent according to Claim [2] 1 in which the L-chain or L-chain fragment is linked to the translocation domain of a clostridial neurotoxin H-chain by a direct covalent linkage.

37. (Thrice amended) An agent according to Claim [2] 1 in which the L-chain or L-chain fragment is linked to the translocation domain of a clostridial neurotoxin H-chain by a covalent linkage which includes [at least one] a spacer region.

39. (Thrice amended) An agent according to Claim 1 in which the lectin is linked to the L-chain or fragment thereof, [and/or] or to the [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain by a direct covalent linkage.

40. (Thrice amended) An agent according to Claim 1 in which the lectin is linked to the L-chain or fragment thereof, [and/or] or to the [molecule or] translocation domain

[with membrane translocating activity] of a clostridial neurotoxin H-chain by a covalent linkage which includes [at least one] a spacer region.

41. (Thrice amended) An agent according to Claim 1 in which the lectin, L-chain or fragment thereof, and [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain are produced as a recombinant fusion protein.

42. (Thrice amended) An agent according to Claim 1 in which the lectin protein has [at least one] an amino acid insertion, deletion, or substitution when compared with the polypeptide sequence of the corresponding native lectin protein, and retains an ability to bind to an oligosaccharide structure having an exposed galactose or N-acetylgalactosamine residue.

43. (Twice amended) An agent according to Claim 42 in which the nucleic acid coding for the lectin protein has [at least one] a nucleotide deletion, insertion [and/or] or substitution when compared with the nucleic acid sequence coding for the corresponding native lectin protein.

46. (Thrice amended) A method for obtaining an agent according to Claim 1 which comprises:- the covalent attachment of a galactose-binding lectin, an L-chain or an L-chain fragment of a clostridial neurotoxin which L-chain or L-chain fragment includes the active proteolytic domain of the L-chain, and a [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain; thereby providing an

agent in which the galactose-binding lectin, L-chain or L-chain fragment, and [molecule or] translocation domain [with membrane translocating activity] of a clostridial neurotoxin H-chain are linked together by a covalent bond.

47. (Thrice amended) A method for obtaining an agent according to Claim 46, wherein the covalent attachment includes [at least one] a spacer region.

51. (Thrice amended) A method of controlling the transmission of sensory information from a primary sensory afferent to a projection neuron by administering to a subject in need thereof an effective amount of the agent of Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus controlling transmission of sensory information.

52. (Thrice amended) A method of controlling the transmission of sensory information from a primary nociceptive afferent to a projection neuron by administering to a subject in need thereof an effective amount of the agent of Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus controlling transmission of sensory information.

54. (Thrice amended) A method of controlling the sensation of pain by administering to a subject in need thereof an effective amount of the agent of Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus controlling the sensation of pain.

57. (Twice amended) A method of alleviating [and/or] or preventing pain which comprises administering to a subject in need thereof an effective dose of the agent according to Claim 1 by a route selected from the group consisting of intrathecal, subcutaneous, and epidural routes, thus alleviating or preventing pain.

Appendix A

Examples of Translocation Function Tests

These papers describe studies of the translocation function of the *Clostridium botulinum* type A neurotoxin. The papers demonstrate that the ability of said neurotoxins to form channels is associated with the presence of a translocation function. To demonstrate this, the authors employed a 50 kDa H-chain fragment representing the NH_2-terminal portion of the heavy subunit (the "H_N fragment").

Shone, C.C., et al., Eur. J. Biochem. 167:175-180 (1987) (Shone et al. II)

Shone *et al.* II used artificial liposomes loaded with potassium phosphate buffer (pH 7.2) and radiolabelled NAD to compare the activity of the intact neurotoxin, the entire H-chain, the LH_N fragment, H_N and the L subunit. In this respect, release of K^+ and NAD from the liposomes correlates with a positive result for channel forming activity and hence translocation activity. K^+release from liposomes was measured using an electrode and NAD release was calculated by measuring the radioactivity in the supernatant. *See* Shone *et al.* II, page 176, col. 1, line 33, through col. 2, line 17.

In more detail, addition of H_N to liposomes suspended in buffer at pH 4.0 - 4.5 caused the release of K^+ into the supernatant. Similar positive results were achieved using the intact neurotoxin, H-chain and LH_N fragment. No release of K^+ was seen using the L-chain subunit alone. A pH effect was observed, with maximal release of K^+ occurring when neurotoxin/ active fragment was added to liposomes at pH 4.5. Release decreased as external pH was

increased and release was undetectable at >pH 6.0. Both the entire toxin and the H_N fragment alone caused release of NAD from liposomes, as evidenced by measurement of >80% of total radioactivity in the supernatant. *See* Shone *et al.* II, "Results" section, pages 176-178.

Blaustein, R.O., et al., FEBS Letters 226:115-120 (1987) (Blaustein et al.)

A different test is illustrated by Blaustein *et al.*, wherein planar phospholipid bilayer membranes are used to test for channel forming activity. In more detail, the entire neurotoxin heavy chain, the H_N fragment, the LH_N fragment and the H_C fragment were tested by the Blaustein *et al* method. Salt solutions on either side of the membrane were buffered at different pH - on the *cis* side, pH 4.7 or 5.5 and on the *trans* side, pH 7.4. Neurotoxin fragments were added to the *cis* side of the membrane and electrical measurements were made under voltage clamp conditions, in order to monitor the flow of current across the membrane (*see* paragraph 2.2, at pages 116-118). Addition of H_N resulted in a steady rate of channel turn-on (*i.e.*, a positive result for channel formation) and, therefore, the presence of a desired translocation activity (*see* paragraph 3 at page 118). As above, it was noted that low *cis* pH is required for channel formation.

Appendix B

Conjugation Examples

Using the building blocks of E (enzyme), T (translocation) and B (Binding), wherein E and T are prepared as a covalently coupled molecule (through a reducible S-S) such that they exist in the order (N-terminus) E-T (C-terminus), the following "agent" permutations are generated by the conjugation method described in the Examples of the present specification:

	Derivatisation of E or T	**Derivatisation of B**	
		1	2
Localisation of coupling agent solely on E	1	B-E-T	B-E-T; T-E-B-E-T
	2	B-E-T; $(B)_2$-E-T	B-E-T; $(B)_2$-E-T; T-E-B-E-T; T-E-B-E(B)-T; T-E-B-E(B-E-T)-T
Localisation of coupling agent solely on T	1	E-T-B	E-T-B-T-E
	2	E-T-B; E-_-$(B)_2$	E-T-B-T-E; E-T-$(B)_2$; E-T-$(B\text{-}T\text{-}E)_2$
Localisation of single coupling agent each on E and T	1 on each	B-E-T; E-T-B; B-E-T-B	E-T-B-T-E; T-E-B-E-T; E-T-B-E-T; T-E-B-T-E

Appendix C

Examples of Tests for the Removal or Reduction of Native Binding Function Resulting from H-Chain Modifications

These papers disclose methods for assessing binding of the H-chain of a clostridial neurotoxin to its target cells, motor neurons. Hence, these methods provide a means of determining whether a modification to the H-chain results in a loss of or reduced native binding affinity of the H-chain for motor neurons.

Shone, C.C., et al., Eur. J. Biochem. 151:75-82 (1985) (Shone et al. I)

Binding of neurotoxin fragments to purified rat cerebrocortical synaptosomes was assessed in competition experiments with radiolabelled intact BoNT/A neurotoxin. Synaptosomes were incubated with radiolabelled intact BoNT/A and trypsinized neurotoxin fragments and following washing steps, the radioactivity of the synaptosomal fraction was determined (*see* page 76, col. 1, line 51, through col. 2, line 5). Reduction of neurotoxin binding is demonstrated by reduced ability of fragments to compete with the labelled intact toxin for binding to the synaposomes.

Trypsin treatment of BoNT/A rapidly reduced the effectiveness of the toxin to compete with radiolabelled, untreated BoNT/A for binding to rat synaptosomes. This decrease in binding was found to correlate with decreased toxicity, however, both binding and toxic activity were lost more rapidly than the rate of cleavage, as assessed by PAGE (see page 79, col. 1, line 17, through page 80, col. 2, line 25). This suggests that rapid tryptic

action, at a site too close to the COOH terminus of the H_C chain for PAGE detection, destroys the neurotoxin binding site and is, therefore, responsible for the rapid loss of toxicity and binding of the BoNT/A neurotoxin (*see* page 82, col. 1, last line, through col. 2, line 8).

Sutton, J.M., et al., FEBS Letters 493:45-49 (2001) (Sutton et al.)

Sutton *et al.* carried out similar competition binding experiments using radiolabelled intact tetanus neurotoxin (TeNT) and unlabelled site-directed (TeNT) mutants. As above, a positive result in the assay is demonstrated by an inability of the mutant fragments to compete with the labelled TeNT for binding to synaptosomes. Sutton *et al.* (*see* page 46, col. 2, lines 6-21) attribute this binding assay to Shone *et al.* I.

Black, J.D., and Dolly, J.O., J. Cell Biol 103:521-534 (1986) (Black and Dolly)

Black and Dolly used electron microscopic autoradiography to visually assess binding of radiolabelled BoNT/A and B neurotoxins at the vertebrate neuromuscular junction, both *in vivo* and *in vitro*.